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Regulation of neuronal functions by the E3-ubiquitinligase protein associated with MYC (MYCBP2)

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The E3-ubiquitinligase MYCBP2 regulates neuronal growth, synaptogenesis and synaptic plasticity by modulating several signaling pathways including the p38 MAPK signaling cascade. We found that loss of MYCBP2 in peripheral sensory neurons inhibits the internalization of transient receptor potential vanilloid receptor 1 (TRPV1) in a p38 MAPK-dependent manner. This prevented desensitization of activity-induced calcium increases and prolongs formalin-induced thermal hyperalgesia in mice. Besides its function in pain perception TRPV1 is also involved in the regulation of neuronal growth. Therefore, the observed effect of MYCBP2 on TRPV1 internalization could be part of the mechanisms underlying its well documented regulatory role in neuronal growth. The clarification of the mechanism is important for the understanding of the different MYCBP2-functions in diverse neuronal subpopulations and species.

Key words: MYCBP2, TRPV1, p38 MAPK, pain, sensory neurons

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The E3-ubiquitin ligase MYC binding protein 2 (MYCBP2) (1) is a giant protein of 510 kDa which regulates several signaling pathways and physiological processes through a multitude of protein binding sites. MYCBP2 has originally been identified as Protein Associated with Myc (PAM) and orthologs have been described in mouse as Phr1, in Zebrafish as Esrom, in Drosophila as Highwire and in *C. elegans* as RPM-1. While MYCBP2 mRNA is found in nearly all human tissues, its expression is exceptionally high in peripheral and central neurons.[1-3] MYCBP2 is upregulated shortly after birth in the cerebellum and hippocampus, a time representing the major synaptogenic period in these structures and correlating with the regulation of neuronal growth by MYCBP2.[2] MYCBP2 has been demonstrated to influence neuronal outgrowth and synaptogenesis by regulating the cAMP,[4,5] Smad4,[6] mTOR[17,8] and p38 MAPK-signaling pathways.[9]

Independently of its well documented role as negative regulator of neuronal growth,[6-9] MYCBP2 also modulates synaptic functions of adult animals. In this regard it has previously been shown that MYCBP2 decreases spinal nociceptive processing by inhibition of adenylyl cyclases, the enzyme which catalyzes the conversion of ATP to cAMP.[3] It is well known that elevated cAMP levels increase neuronal excitability mainly through protein kinase A (PKA)-dependent phosphorylation of ion channels as well as PKA- and cAMP-response-element-binding protein (CREB)-mediated changes in gene expression.[10] Accordingly, down-regulation of MYCBP2 in the spinal cord has been shown to cause an increase in the cAMP-synthesis and, therefore, nociceptive behavior.[3]

Regulation of Receptor Trafficking by MYCBP2

MAPK kinase kinase 12 (MAP3K12, also known as dual leucine zipper bearing kinase, DLK is an ubiquitylation target of MYCBP2.[9] MAP3K12 activates the p38 MAPK-pathway. Interestingly, p38 MAPK regulates the TRPV1-translation, TRPV1 axonal transport, TRPV1-recruitment to the nociceptor[11] and the

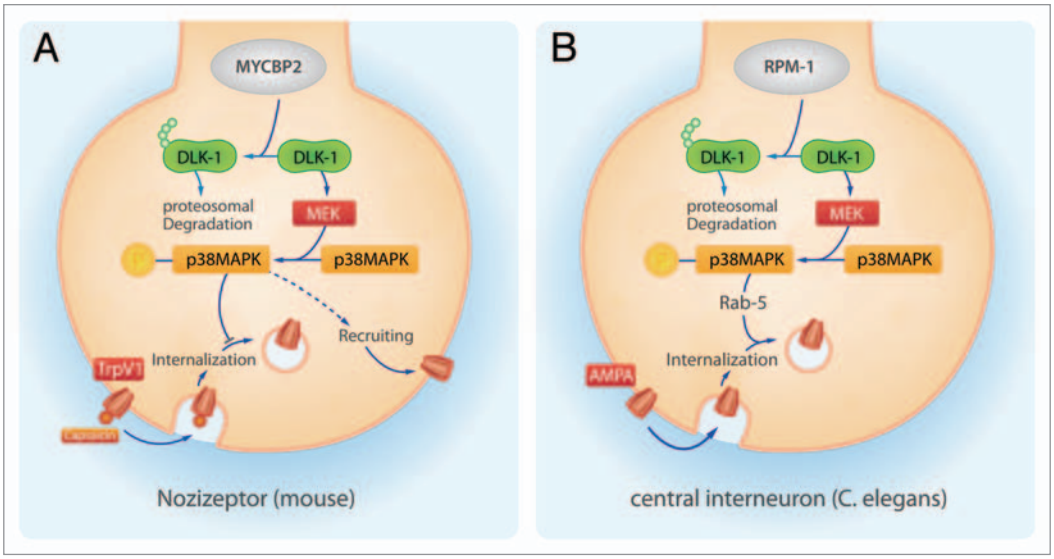

Figure 1. Schematic of the MYCBP2/p38MAPK signaling pathway regulating receptor internalizations. MYCBP2 and its ortholog RPM-1 inhibit the p38 MAPK cascade by targeting MAP3K12 for proteosomal degradation. Activated p38 MAPK prevents the internalization of the TRPV1 at nociceptors of mice, while facilitates at central interneurons of *C. elegans* the internalization of AMPA receptors.

production of important proinflammatory cytokines like TNFα or IL-1 [12] making p38 MAPK an important factor for sensibilization procedures of nociceptive neurons. In this regard it was not surprising that loss of MYCBP2 in peripheral sensory neurons caused an increased nociceptive behavior in an animal model for inflammatory pain. We found that MYCBP2-deficiency caused a constitutive p38 MAPK activation which, surprisingly, prevented activity-induced internalization of the transient receptor potential vanilloid receptor 1 (TRPV1) leading to the p38 MAPK-mediated prolonged thermal hyperalgesia.

The observed inhibition of TRPV1-internalization by loss of MYCBP2 contrasts the previously described role of p38 MAPK in receptor internalization in other mammalian systems.[13-15] Even though an inhibition of endocytosis would augment the increased translation and peripheral transport of TRPV1 by activated p38 MAPK. However, it was demonstrated that p38 MAPK activation is necessary for μ-opioid receptor endocytosis and is sufficient to trigger constitutive internalization of μ-opioid receptor in the absence of agonists.[14] These contrasting findings suggest that p38 MAPK is able to fulfil different roles in receptor trafficking. This assumption is supported by the finding that phosphorylation of epidermal growth factor (EGF)-receptor between amino acids 1002 and 1022 mediates stress-induced internalization[15] while EGFR phosphorylation at serine 1046 and 1047 by p38 MAPK mediates ubiquitylation and degradation of already internalized EGF receptor but not internalization itself.[16,17] Therefore it is not completely surprising that the loss of MYCBP-ortholog RPM-1 facilitates AMPA receptor internalization in *C. elegans*.[18] Additionally it should be noted that RPM-1-mediated increased AMPA receptor internalization is found in central interneurons, while the prevention of MYCBP2-mediated TRPV-1 internalization was observed in peripheral sensory neurons (**Fig. 1**).

Notably, besides the functional role of TRPV1 in nociceptive processing this receptor is also involved in calcium-mediated neuronal growth.[19,20] Therefore, it can be hypothesized that the described inhibition of TRPV1 internalization by the loss of MYCBP2 might be one of the mechanism by which MYCBP2 regulates neuronal growth.

Neuron-Specific MYCBP2 Functions

The possibility that MYCBP2 fulfils diverse functions in the different neuronal subpopulations has already been raised previously. Grill et al. hypothesized that two parallel MYCBP2-mediated signaling pathways regulate neuronal growth in *C. elegans*.[21] This assumption was partly based on the findings that loss of the MYCBP2-ortholog RPM-1 in motor neurons causes less synapses with disorganized structures[9,22] while RPM-1-deficiency in mechanosensory neurons leads to instable synapse branching and prolonged axonal growth.[23] Furthermore MYCBP2-mediated growth of cortical axons in mice is MAP3K12-independent while MYCBP2-dependent axonal growth of spinal cord motor neurons and sensory dorsal root ganglion (DRG) neurons was regulated by p38 MAPK-mediated alterations in microtubule stability.[24,25]

Diverse roles of PAM in specific neurons are also supported by the reported phenotypes of general and conditional MYCBP2 knockout mice (**Table 1**). The ubiquitous deletion of MYCBP2 leads to neuronal dysfunction and lethality in vertebrates while mutations in invertebrates are viable. The general MYCBP2 deletion in mice causes the development of markedly narrower phrenic nerves with fewer axons, resulting in incomplete innervations of the diaphragm. The resulting respiratory failure leads to postnatal lethality in mice[24,26] while, due to their physiological differences, invertebrates show motoric disturbances but are still viable.[9,27] Specific deletion of MYCBP2

in motor neurons resulted in an phenotype which allowed some mice to be viable pointing to a possible involvement of non-neuronal expressed MYCBP2.[24] Fittingly, specific MYCBP2-deletion in sensory neurons did not influence viability.[25] However, it remains unclear why conditional MYCBP2 deletion using the Nestin-promotor, leading to a MYCBP2-deficiency in all neuronal and glia cells, causes prenatal lethality.[25] Taken together, the data suggest strongly that MYCBP2 fulfils several roles in regulating neuronal functions and future studies should therefore focus on the specific roles of MYCBP2 in the different cell types.

Table 1. Viability of MYCBP2-knockout animals

Species	Tissue	Viability	Reference
C. elegans	ubiquitous	viable	22, 23
D. melanogaster	ubiquitous	viable	27
D. rerio	ubiquitous	lethal	28
M. musculus	ubiquitous	lethal	24, 26
	neuronal	lethal	25
	Motor neurons	partially viable	24
	Sensory neurons	viable	25

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